# COMPARATIVE CYTOLOGICAL EXAMINATIONS OF THE PARAVERTEBRAL GANGLIA OF TADPOLES AND FULL-GROWN FROGS

I. HORVÁTH

Institute of General Zoology and Biology, Attila József University, Szeged
(Received October 3, 1966)

The light- and electronmicroscopic examinations have resulted in a more perfect cognition of the structure of nerve cells. Already in the early years of the century, the research workers observed granules of different size in the neurons, even without staining. These granules were classified into two groups: the fine granuler lipofuscin pigment stained yellowish-brown was classed into one of the groups, and the rough granular melanin pigment of brownish-black colour into the other one.

The opinion of scientists concerning the occurrence of pigments is not uniform. According to some of them, melanin is restricted but to certain cell groups of the central nervous system *(Nucleus niger, Locus coeruleus, nucleus of the dorsalis vagus);* in other *opinions* (KUNTZ, 1934; BARGMANN, 1948; STRONG and ELWYN, 1948, and LARSELL, 1951), it occurs in men also in the spinal and sympathetic *ganglia.* The latter opinion is not confirmed by the recent examinations of SULKIN (1953). According to him, the dark staining in the sympathetic cells is caused by lipofuscin present there, the chemical nature of which differs strongly from that of melanin.

About the chemistry of pigments we know but little. BIELSCHOWSKY (1928) and LEHNARTZ (1942) supposed that in the nerve cells melanin developes through the oxidation of dihydroxiphenilalanin (DOPA) and o-quinon differring, anyhow, substantially from the melanin of skin (LE GROS CLARK, 1945).

The yellowish-brown lipofuscin granules arise, however, in the opinion of LILLIE (1948) and PEARSE (1953), in the way of a progressive oxidation from a precursor of lipid content. Pigment substances, thus also the *„ceroid"* pigment in liver, are considered by PEARSE as middle products of oxidation processes. And SPIEGEL and ADOLF (1822) try to derive the yellow pigment from the black one.

Completing the light microscopic pigment examinations with electronmicroscopic ones, we may conclude that the imbedded substances of the vegetative nerve cells, or at least a part of them, can be classed into the group of lysosomas showing an acid phosphatase activity, changing, growing, and achieving possibly the size of more microns (TAXI, 1965). The identity of the genesis of lipofuscin and lysosoma is proved by a series of experiments verifying that the lysosoma pigment transforms, under the influence of a radioactive irradiation, into lipofuscin turning, however, back into lysosoma after the irradiation that stopped, again showing an acid phosphatase activity.

## Material and method

I have examined the species *Pelobates fuscus fuscus, Rana ridibunda,* and *Bufo viridis* in different states of their development. The *ganglia* of the limiting fascicle of one side, dissected from the animals, have been fixed in a pH 7,4 osmium solution pufferred according to MILLONIG; and the *ganglia* of the limiting fas-

cicle of the other side in formalin. The *ganglia* fixed for the electronmicroscopic examinations were embedded into Araldit and the slides examined by an electronmicroscope TESLA 242 D. The histochemical demonstration of pigments took place with SCHMORL's method. In addition, also stainings with Sudan black, alkali tetrasolium, Toluidin-blue, Gallo-cyanin, Acridin-orange, Janus-green, and neutralred were carried out for demonstrating collectively the other *organella* of cell, as well.

## Examination of the ganglia of the sympathetic limiting fascicle

I have examined the ontogenesis of the vegetative nervous system of tadpoles for several years. At the tadpoles of different development I have followed with attention the formation of *ganglia* of the *truncus sympathicus* (HORVÁTH, 1965): from the sympathicoblast through the sympathicocyte till the fully developed *ganglion*. The endoamitotical division of the sympathicoblast cells can well be observed by a light microscope. The 4—7 $\mu$ thick slides of the vegetative *ganglia*, prepared in series, were treated parallel with reagents and stains of different pH. From the methods used by me for demonstrating lipofuscin, although not specific ones, compared with literary data known by me, the conclusion can be drawn that the pigments observed belong to the group of lipofuscin. That result is confirmed also by the electronmicroscopic examinations where I could observe in some of them, apart from the polynucleosity of the sympathicoblast cells, some pigments of different size, stained well by osmium. Considering that the research objects were tadpoles in different states of their development, these results may supposedly serve as a basis for the genesis of lipofuscins, *resp.* lysosomalike cell components unknown, as yet. In the course of my further examinations, I should like to clear up approximatively the G. E. R. L. functional system set up by NOVIKOFF in 1964, trying to discover a connection between GOLGI's apparatus, the endoplasmatic *reticulum*, and lysosoma. In my present paper I want to get some conclusions concerning the time of appearance of the pigment and its role inside the cells.

The sympathicoblasts deriving from the neural crest form smaller or bigger groups in the line of the limiting fascicle, suitable for an endomitotic division. The sympathicocytes produced in the course of division contain, in contradiction to the giant sympathicoblasts, not more than two *nuclei* and but a single appendix. In this way, the sympathicocytes can be divided once more. The lipofuscin pigments can be observed in a great quantity first of all in the sympathicoblasts of 24—32 $\mu$ capable of being divided endoamitotically more times (Fig. 1). And also the fusion of these granules of 0,01—0,5 $\mu$ can be observed. The granules can supposedly fuse and, surrounded by a simple membrane, turn into lysosomas (Fig. 2). In the other ganglia of the more developed tadpoles the amount of lipofuscin pigments falls to a minimal one and, in the same time, in a bigger amount, also the storage of *mitochondria* may be observed, apart from the tigroid granules. This picture can be seen in the *ganglia* of the limiting fascicles of one year old frogs (Figs. 3—4). In the *ganglia* of older animals, at

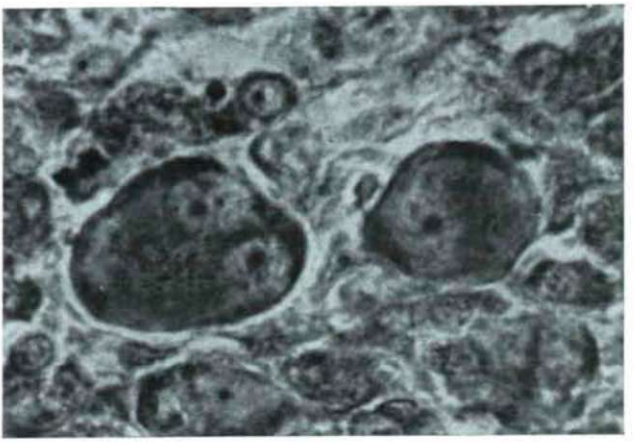

Fig. 1. *Pelobates fuscus* (tadpole): Large amount of lipofuscin granules in the cytoplasm of the sympathicoblast dividing endoamitotically. SCHMORL's method. Microphotograph, x 675.

an advanced degerenation, the cells perish after a major increase of the tigroid granules (Fig. 5). In these cases the *nucleus* of excentric site shrinks considerably, and a number of lipofuscin granules take place, in a group, at the pole facing the *nucleus*.

From the above-mentioned accumulation of a major amount of pigments in the giant sympathicoblast cells of tadpoles, and from the occurrence of a comparatively smaller number of pigments in the *ganglia* of the fully developed frogs the conclusion may be drawn that the biochemical processes in cells must have been carried out, even if in a lower degree, supposedly in the presence of other *organella* producing and storing energy. The proportion of the comparative amounts of tigroid granules and *mitochondria* is worth mentioning, as well. In the degenerating sympathicoblast cells, even in spite of the high degree of protein synthesis, the number of *mitochondria* is very low compared with that of the fully developed *ganglia*. The quantitative difference of tigroid granules may change in a high degree depending upon the functional state, therefore, I don't want to make any comparison in this field.

After the conclusions made known above I have to mention some literary data, as well, concerning the intracellular role of pigments. It is generally accepted that the amount of yellow pigments increases with the age, at a lasting output, and in the case of some diseases. Yet we can find several ideas opposite to that statement. According to KUNTZ (1932) the pigmentation of human vegetative nerve cells may be observed in the middle period of his life without influencing by it the cell productivity. WHITE (1887, 1889) asserts, alluding to animals on a lower degree of phylogenesis, that the vegetative cells are not pigmented, as yet. The pigments appearing later result in a sure decrease of functioning. SCHAEFER (1893) considers the pigmentation not as a degenerative phenomenon but as a function-increasing one. According to HYDÉN (1950),

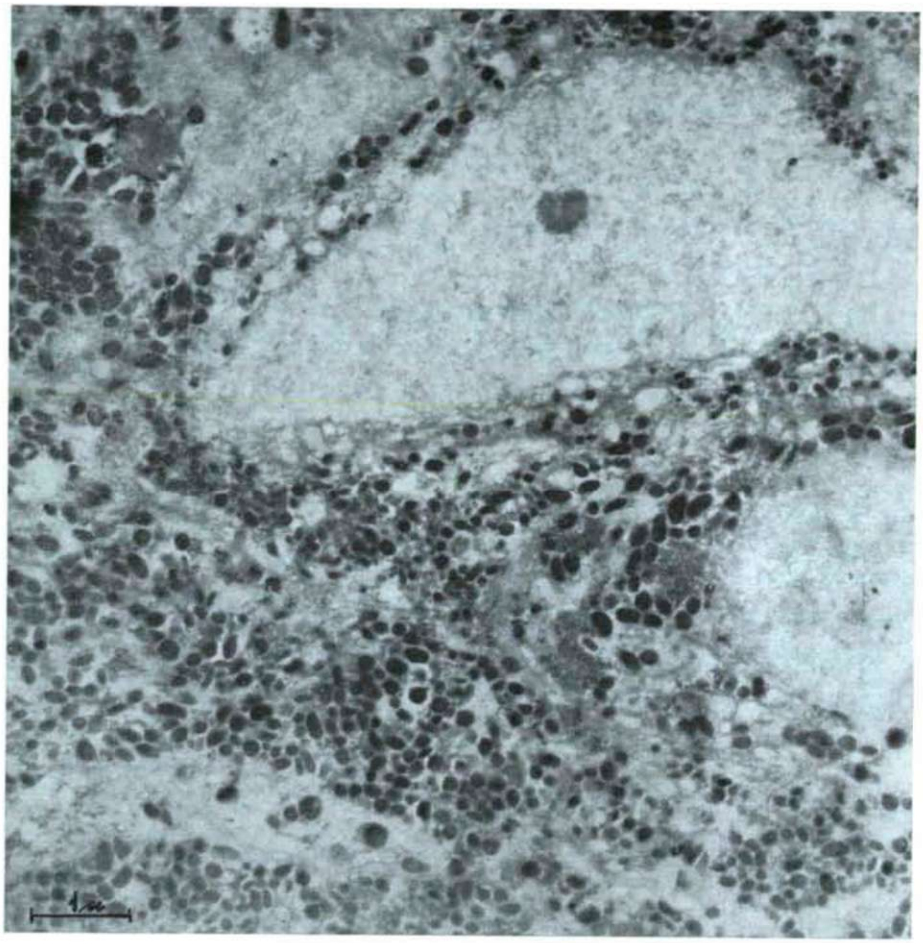

Fig. 2. *Pelobates fuscus* (tadpole): An electronmicroscopic photograph about a binuclear cell of a sympathicoblast in which scattered lipofuscin granules are taking place. Araldit embedding. Magnified x 15 050.

after the yellow pigments had appeared, the cells become differentiated, as proved also by the presence of the cytoplasma nucleotide demonstrated beside the pigments. HYDÉN and LINDSTRÖM (1950) assert also on the basis of their examinations concerning the mass determination by roentgendiffraction that the pigment accumulation at the advancing age results in a further chemical organization of neurons. It may be supposed on the basis of the histochemical examinations of GEDIGK and BRONTKE (1956), as well, that the lipopigment plays a peculiar role in cell metabolism. We must doubtless attribute a considerable role to the pigments in the synthesis of the high degree metabolism taking place in the sympathicoblasts of tadpoles.

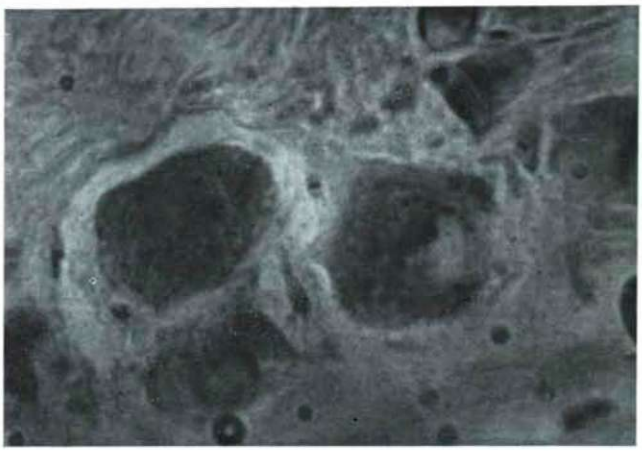

Fig. 3. *Bufo viridis:* Tigroid granules in the nerve cells of *Ggl. sympathicum* VI. Toluidin-blue staining. Microphotograph, x 675.

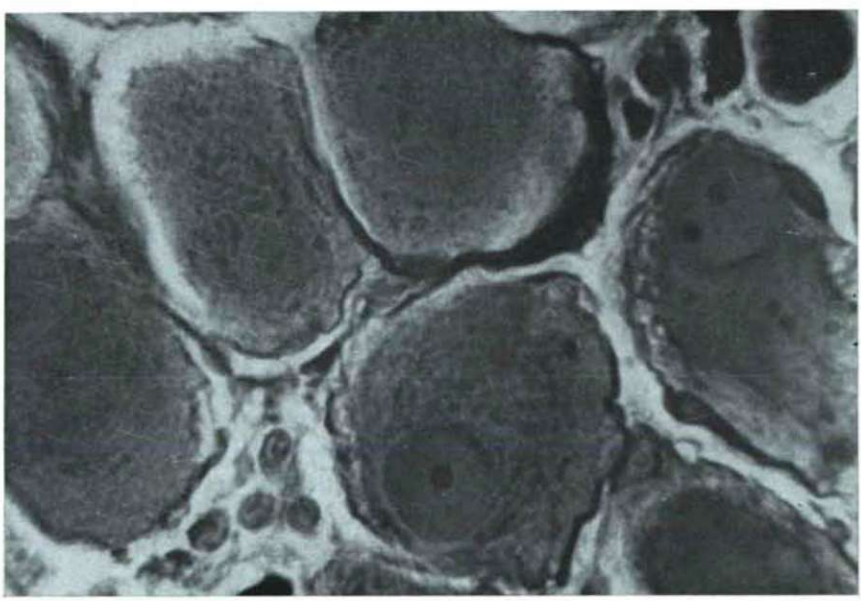

Fig. 4. *Rana ridibunda:* Demonstration of *mitochondrium* in the *ganglia* of *Ggl. sympathicum* VI. Stained by Janus-green B. Microphotograph, x 1054.

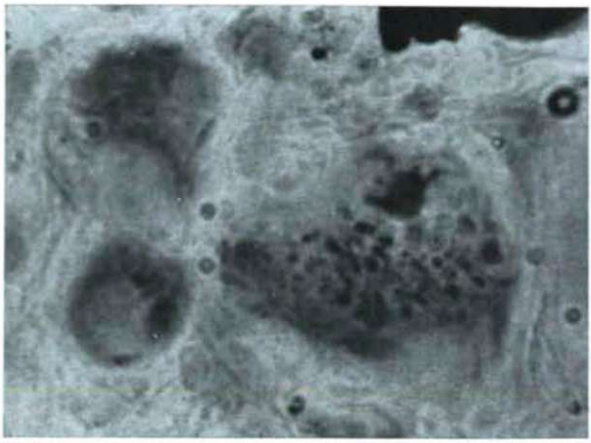

Fig. 5. *Rana ridibunda:* Nissl substance accumulated in the degenerating nerve cells of an old frog. (From *Ggl. sympath.* VI). Gallocyanin-chrome-aluminous method. Microphotograph, x 675.

There are differing opinions concerning the genesis of the pigments of nerve cells, as well. DOLLEY (1917) derives the pigment from the nucleic subtance. According to MONROY (1934/35), however, the granules of pigment develop from the *mitochondria.* Therefore, several researchers are occupied by clearing the raised problems. Partly I should transgress the framework of this paper, partly also I should be constrained to build upon mere suppositions if I tried to answer these important questions.

## Summary

I can summarize the results of my comparative cytological examinations carried out on tadpoles of different development *(Rana ridibunda, Pelobates fuscus fuscus, Bufo viridis)* and on fully developed frogs, as follows.

1. I could demonstrate a large amount of lipopigments taking sporadically place in the giant sympathicoblast cells of tadpoles in development.

2. The amount of lipopigments decreases gradually from the young neurocytes till the full-grown nerve cells, instead of them an increase of the number of the tigroid granules and that of *mitochondria* my be observed.

3. In the vegetative *ganglia* of older animals the lipofuscin granules occur forming lesser groups in the cytoplasm opposed to the *nucleus.*

4. In my opinion, the large amount of the pigment granules of the sympathicoblast cells may have an absolutely important role in cell metabolism.

5. We may conclude the degeneration of nerve cells not so much from the pigment increase but more from the inrease of the number of tigroid granules and from the decrease of that of *mitochondria.*

## References


BARGMANN, W. (1948): Histologie und mikroskopische Anatomie des Menschen. Bd. I. Stuttgart: Georg Thieme.

BIELSCHOWSKY, M. (1928): Nervengewebe. In: Handbuch der mikroskopischen Anatomie des Menschen. Bd. IV/1. Berlin: Springer.

DOLLEY, D. H. (1917): The recovery from depressions in the Purkinje cell and the decline to senility of depression, with an incidental histogenesis of abnormal pigmentation. J. comp. Neurol. 28, 465—493.

GEDIGK, P.—BRONTKE, E. (1956): Über den Nachweis von hydrolytischen Enzymen in Lipopigmenten. Z. Zellforsch. 44, 495—518.

HORVÁTH, I. (1965): Neurohistologische Untersuchungen an den paravertebralen Ganglien von Kaulquappen. Acta Biol. XI. fasc. 3—4, 257—264.

HYDÉN, H. (1950): Spectroscopic studies on nerve cells in development, growth and function. In: Genetic Neurology, edit. P. Weiss, 177—193.

HYDÉN, H.—LINDSTRÖM, B. (1950): Some physicochemical properties of the yellow pigment in the nerve cells. Quoted after Hydén.

KUNTZ, A. (1932): The sympathetic nerve cells. In: Special Cytology, edit. Cowdry, 1422—1444. New-York: Hoeber.

KUNTZ, A. (1934): The autonomic nervous system. Philadelphia: Lea and Febiger.

LARSELL, O. (1951): Anatomy of the nervous system. 2nd edition. New-York: Appleton-Century-Crofts.

LE GROS CLARK, W. E. (1945): The tissues of the body. 2nd edition. Oxford: Clarendon Press.

LEHNARTZ, E. (1942): Einführung in die chemische Physiologie: 5. Aufl. Berlin: Springer.

LILLIE, R. D. (1948): Histopathologic technic. Philadelphia: Blackiston Co.

MONROY, A. (1934/35): I condriosomi e i lipoidi nelle cellule del simpatico e la loro impartanza nelle genesi del pigmento giallo. Anat. Anz. *79*, 383—390.

NOVIKOFF, A. (1964): *GERL*, its form and function in neurons of rat spinal ganglia. Biol. Bull., 127, 358.

PEARCE, A. G. E. (1953): Histochemistry, theoretical and applied. London: J. A. Churchill.

SCHAFER, E. A. (1893): The nerve cell considered as the basis of neurology. Brain 16, 134—169.

SPIGEL, E. A.—ADOLF, M. (1922): Die Ganglien des Grenzstranges. Arb. neurol. Inst. Univ. Wien 23, 67—177.

STRONG, O. S.—ELWYN, A. (1948): Human Neuroanatomy. 2nd edition. Baltimore: Williams and Wilkins.

SULKIN, N. M. (1953): Histochemical studies of the pigments in human autonomic ganglion cells. J. Geront. 8, 435—445.

TAXI, J. (1965): Contribution à l'étude des connexions des neurones moteurs dy système nerveux autonome. Ann. Sci. Naturelles, Zool, Série 12, Tome VII. 413—674.

WHITE, W. H. (1887): On the histology and function of the mammalian superior cervical ganglion. J. Physiol. 8, 66—78.

WHITE, W. H. (1889): Further observations on the histology and function of the mammalian sympathetic ganglia. J. Physiol. 10, 341—357.